



Legend to Supplementary information 4

Phosphorylation-deficient form of Nbs1 undergoes quantitative recruitment to γ -H2AX-containing chromosomal areas in Mdc1-proficient but not in Mdc1-depleted cells.

(A, B) U-2-OS cells stably expressing the phosphorylation-deficient (3xA) form of Nbs1-2GFP (see Methods) were treated with control (A) or Mdc1-targeting (B) siRNA for 96 h and microirradiated. After 10 min., the cells were pre-extracted with detergent-containing buffer (see Methods), fixed and immunostained with phospho-specific antibodies to γ -H2AX. Arrows indicate the laser movement during microirradiation.

Scale bars = 10 μ m.

(C) U-2-OS cells expressing the wild-type (wt) or phosphorylation-deficient (3xA) forms of Nbs1-2GFP were microirradiated and subjected to the kinetic measurement of their recruitment to DSB (see Methods). The graph integrates the data from 10 cells for each setting and shows the fold of increase of relative GFP-associated fluorescence in the microirradiated areas during the first 10 min after the laser treatment.